with objections or requirements as to form in accordance with 37 C.F.R. 1.116(a), and that the present amendment places the application in condition for allowance or in better form for appeal.

In the Final Office Action dated February 3, 2000, the Examiner rejected claims 1-20, and 26 under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regards as the invention. Claims 30, 31 and 34 were rejected under 35 U.S.C. §102(e) as being anticipated by Hartley et al. Claims 32 and 33 were objected to and Claim 35 was allowed.

Claims 1-20 and 26 contained the recitation "gene expression regulatory element", which the Examiner characterized as indefinite. In the Final Office Action, however, the Examiner indicates at page 3 that transcriptional control is sufficiently taught by the Applicants and that it is not indefinite. Accordingly, Applicants have amended claims 1 and 26 replacing "gene expression" with "transcription".

Amended claims 1 and 26 notwithstanding, Applicants respectfully urge that "gene expression" is more than sufficiently enabled by Applicants' disclosure. Specifically at page 17, line 16 to page 19, line 6:

> As used herein, the term "regulatory element" refers to a genetic element that controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element that facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements are splicing signals, polyadenylation signals, termination signals, etc. (defined *infra*).
>
> Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription [Maniatis, T. *et al., Science* 236:1237 (1987)]. Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect, and mammalian cells and viruses (analogous control elements, i.e., promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what

cell type is to be used to express the protein of interest. Some eukaryotic promoters and enhancers have a broad host range while others are functional in a limited subset of cell types [for review, *see* Voss, S.D. *et al., Trends Biochem. Sci.,* 11:287 (1986) and Maniatis, T. *et al., supra* (1987)]. For example, the SV40 early gene enhancer is very active in a wide variety of cell types from many mammalian species and has been widely used for the expression of proteins in mammalian cells [Dijkema, R. *et al, EMBO J.* 4:761 (1985)]. Two other examples of promoter/enhancer elements active in a broad range of mammalian cell types are those from the human elongation factor 1α gene [Uetsuki, T. *et al, J Biol. Chem.,* 264:5791 (1989), Kim, D.W. *et al., Gene* 91:217 (1990) and Mizushima, S. and Nagata, S., *Nuc. Acids. Res.,* 18:5322 (1990)] and the long terminal repeats of the Rous sarcoma virus [Gorman, C.M. *et al, Proc. Natl. Acad Sci. USA* 79:6777 (1982)] and the human cytomegalovirus [Boshart, M. *et al, Cell* 41:521 (1985)].

As used herein, the term "promoter/enhancer" denotes a segment of DNA that contains sequences capable of providing both promoter and enhancer functions (i.e., the functions provided by a promoter element and an enhancer element, see above for a discussion of these functions). For example, the long terminal repeats of retroviruses contain both promoter and enhancer functions. The enhancer/promoter may be "endogenous" or "exogenous" or "heterologous." An "endogenous" enhancer/promoter is one which is naturally linked with a given gene in the genome. An "exogenous" or "heterologous" enhancer/promoter is one which is placed in juxtaposition to a gene by means of genetic manipulation (i.e., molecular biological techniques) such that transcription of that gene is directed by the linked enhancer/promoter.

The presence of "splicing signals" on an expression vector often results in higher levels of expression of the recombinant transcript. Splicing signals mediate the removal of introns from the primary RNA transcript and consist of a splice donor and acceptor site [Sambrook, J. *et al., Molecular Cloning.- A Laboratory Manual,* 2nd ed., Cold Spring Harbor Laboratory Press, New York (1989) pp. 16.7-16.8]. A commonly used splice donor and acceptor site is the splice junction from the 16S RNA of SV40.

Eukaryotic expression vectors may also contain "viral replicons" or "viral origins of replication." Viral replicons are viral DNA sequences that allow for the extrachromosomal replication of a vector in a host cell expressing the appropriate replication factors. Vectors that contain either the SV40 or polyoma virus origin of replication replicate to high copy number (up to 104 copies/cell) in cells that express the appropriate viral T antigen. Vectors that contain the replicons from bovine papillomavirus or Epstein-Barr virus replicate extrachromosomally at low copy number (~100 copies/cell).

And at page 30, line 23 to page 31, line 1:

"c) Modification of Expression Vectors

As discussed above, pUNI vectors are used to transfer a gene of interest into a suitably modified vector via site-specific recombination. The modified vectors or host vectors used in the Univector Fusion System are referred to as pHOST vectors. PHOST vectors are generally **expression vectors (*e.g.* plasmids)** [emphasis added] which have been modified by the insertion of a sequence-specific recombinase target site (*e.g.* a lox site). However, the pHOST can comprise any regulatory sequence desired for manipulation of nucleic acids.

Continuing on page 31, at line 15:

To generate expression vectors intended to generate transcriptional fusions (i.e., pHOST does not contain a vector-encoded protein domain), a sequence-specific recombinase target site is placed after (i.e., downstream of) the start of transcription in the host vector. This is easily accomplished using synthetic nucleotides comprising the desired sequence-specific recombinase target site. In designing the oligonucleotide comprising the site-specific recombinase target site, **care is taken to avoid introducing an ATG or start codon that might initiate transcription inappropriately**. [emphasis added]

That is, expression requires the lack of in-frame stop and start codons, not just transcription promoters.

At page 37, line 3, *et seq.* :

It is clear that a variety of reconstituted regulatory elements can be employed to achieve detectable directional cloning. **For example, reconstituted regulatory elements that find use in the present invention include but are not limited to, promoters, repressors, operators, enhancers, enzyme recognition sites, selectable markers and conditional origins of replication, among others.** [emphasis added] It is also contemplated that the reconstituted regulatory element may comprise a negative selection capability, such that fragments cloned in an undesired orientation reconstitute the regulatory element and are selected against. One skilled in the art will recognize the wide range of regulatory elements and applications that can be applied to this system.

To demonstrate the effectiveness of the above approach, the *lac* operator was employed to direct directional subcloning events. Luria and colleagues observed in

> the early 1960s thatphage carrying the binding site for the *lac* repressor, *lacO*, could induce the **expression** [emphasis added] of the endogenous *lacZ* gene by titrating out a limited number of repressor genes.[....]
>
> This observation was taken advantage of by the methods of the present invention, whereby the 3' half of a *lacO* site was placed on a pUNI vector (i.e., pUNI 30). The *lacO* derivative used was a symmetrical 20 bp site that has an Eco47III site at the center. To utilize this method of cloning PCR derived material, primers were made corresponding to the *SKP*1 GENE. A 10 bp sequence corresponding to the 5' half of the symmetrical *lacO* sequence (shown in Figure 22B) was added to the 5' end of the 3' primer. Figure 22B shows this strategy, whereby primer A (5') and B (3') are used to amplify the gene of interest. The 5' end of primer B contains a half *lacO* site which subsequently becomes the 3' end of the PCR fragment indicated in the Figure. After ligating the PCR fragment into linearized pUNI 30 containing the other half of *lacO*, an intact *lacO* site is reconstituted and, in *lac*+ cells results in **induction of endogenous B-galactosidase** [emphasis added] and production of blue colonies in the presence of X-gal.

The foregoing excerpts from Applicants' specification easily enable one of ordinary skill in the art to both identify and use "gene expression" regulatory elements in the present invention.

The Examiner states in the Final Action (p. 3) that "[a]pplicant simply has not provided the teachings necessary for the skilled artisan to obtain transcriptional control of the nucleic acid of interest with regulatory elements other than promoters once it has recombined into the second nucleic acid construct." Applicants' respectfully disagree and submit that the foregoing excerpts from Applicants' specification teach numerous regulatory elements, not just promoters, including but not limited to, promoters, repressors, operators, enhancers, enzyme recognition sites, selectable markers, conditional origins of replication, among others, and particularly teaching the **gene expression** regulator *lacO*.

Claims 30, 31 and 34 were rejected under 35 U.S.C. §102(e) as being anticipated by Hartley et al. Hartley, however, fails to teach or suggest the use of recombinases provided by the host cell in vivo to carry out the recombination reaction for cloning a gene of interest. Harltey teaches the use

of commercially available recombination enzymes that are added to *in vitro* recombination reactions. Hartley then transforms host cells with the reaction products and selects the host cell colonies for the desired phenotype.

The Examiner states that Hartley teaches "that the recombinase may be supplied either as a protein or a construct which allows expression of the recombinase is transformed in the cell." Providing a construct with which to transform a host cell so that the transformed cell expresses a recombinase, however, does not anticipate providing a host cell that constitutively expresses a recombinase. A DNA construct cannot anticipate a living cell.

Applicants reading of the Hartley '732 reference failed to identify the teaching that the recombinase may be supplied as a construct with which to transform a cell for expression of a recombinase. Applicants respectfully request clarification by the Examiner with a cite to specific columns and lines in the '732 reference providing the relied upon teaching.

In contrast to the Examiner's reading of the '732 reference, Applicants' respectfully believe that Hartley teaches away from Applicants' invention with respect to the source of the recombinase. For example, at col. 7 line 60: "[s]uch purposes include in vitro recombination of DNA segments and in vitro or vivo insertion or modification of transcribed, replicated, isolated or genomic DNA or RNA." That is, recombination occurs only in vitro.

At col. 13, line 67 and col. 14 line 1: "Cre is commercially available."

At col. 14, line 62: "The integration reaction mediated by integrase and IHF works in vitro, with simple buffer containing spermidine."

At col. 15, line 33: "Other site-specific recombinases similar to [lambda] Int and similar to P1Cre can be substituted for Int and Cre. Such recombinases are known. In many cases the

purification of such other recombinases has been described in the art. In cases when they are not known, cell extracts can be used or the enzymes can be partially purified using procedures described for Cre and Int."[emphasis added]

At col. 16, line 20: "The amount of recombinase which is added to drive recombination can be determined by using known assays. Specifically, titration assay is used to determine the appropriate amount of **purified** recombinase enzyme or the appropriate amount of an extract." [emphasis added]

In each of the foregoing citations from the '732 reference, the clear suggestion or teaching is the use of purified or even commercially available recombination proteins in vitro to achieve recombination. Thus, Hartley teaches away from providing a host cell that constitutively expresses a recombinase to supply the recombinase for recombination in vivo. Certainly, none of the above citations teaches or suggests the use of an endogenous supply of recombinase supplied by the host cell to achieve recombination in vivo.

Therefore, actually teach away from Applicants' invention. In each of Hartley's six examples, the recombination protein is added in vitro prior to transformation of a host cell:

EXAMPLE 1

At col. 20, line 13: Part I: About 75 ng each of pEZC602 and PEZC629 were mixed in a total volume of 30 ul of Cre buffer"

EXAMPLE 2

At col. 22, line 14: One microliter aliquots of each supercoiled plasmid (about 50 ng crude miniprep DNA) were combined in a ten microliter reaction containing equal parts lambda integrase buffer [....] and Cre recombinase buffer [....], two units of Cre recombinase, 16 ng integration host

factor and 32 ng lambda integrase. After incubation at 30 C for 30 minutes and 75 C for 10 minutes, one microliter was transformed into competent *E. Coli* strain DH5."

EXAMPLE 3

At col.24, line 58: "Pairs of plasmids were mixed and reacted with Cre, Int., Xis, and IHF, transformed into *E. Coli* competent cells..."

And at col 25, line 35: "Pairs of plasmids were recombined in vitro using Int., Xis, and IHF"

EXAMPLE 4

At col 26, line 39: "One hundred nanograms of plasmids were mixed in buffer [...] containing Int (43.5 ng) , Xis (4.3 ng) and IHF (8.1 ng) in a final volume of 10 ul. Reactions were incubated for 45 minutes at 25° C., 10 minutes at 65° C., and 1 ul was transformed into *E. Coli* DH5)."

EXAMPLE 5

At col. 28, line 35: "Eight microliter reactions containing the same buffer and proteins Xis, Int and IHF as in previous examples were incubated for 45 minutes at 25° C., then 10 minutes at 75° C., and 1 ul aliquots were transformed into DH5 (i.e. dam+) competent cells, as presented in table 6."

EXAMPLE 6

At col. 30, line 38: "Unpurified PCR products (about 30 ng) were mixed with the gel purified, linear rf1, rf2 or rf3 cloning vectors (about 50 ng) in a 10 ul reaction containing 1 x React 4 buffer (LTI) and 1 unit UDG (LTI). After 30 minutes at 37° C., 1 ul aliquots of each reaction were transformed into competent *E. coli* DH5 cells (LTI) and plated on agar containing 50ul/ml kanamycin."

In none of the preceding examples did Hartley use a recombination protein supplied in vivo by a host cell constitutively expressing the recombination protein. In fact, Hartley explicitly warns against in vivo recombination at col. 5, line 48: "***[t]he present recombinational cloning method possesses several advantages over previous in vivo methods. Since single molecules of recombination products can be introduced into a biological host, propagation of the desired Product DNA in the absence of other DNA molecules (e.g., starting molecules, intermediates, and by-products) is more readily realized. Reaction conditions can be freely adjusted in vitro to optimize enzyme activities. DNA molecules can be*** [sic] ***incompatible with the desired biological host (e.g., YACs, genomic DNA, etc.), can be used. Recombination proteins from diverse sources can be employed, together or sequentially.***" [emphasis added]. This paragraph strongly teaches away from Applicants' invention, because Applicants claim a method that uses an endogenous recombination protein supplied by a host cell to clone a gene of interest in vivo, whereas Hartley teaches that such in vivo recombination for cloning is undesirable.

In his Background section, Hartley admits to prior art that uses in vivo recombination proteins, such as Palazzolo et al., and Posfai et al., but then distinguishes his invention from them.

Furthermore, Palazzolo is directed to the in vivo excision of cloned cDNA from a phage lambda vector in *E. coli* using endogenous Cre recombinase, and subsequent amplification, not to the cloning of the cDNA in the first place. Posfai is directed to the integration of a vector into the genome of a cell using the cell's endogenous recombination protein and then cloning the genomic DNA. Neither of these references disclose or suggest the constructs of Applicants' invention.

Claim 3 contained the recitation "wherein said nucleic acid of interest comprises a fusion peptide" which the Examiner characterized as indefinite. The Examiner correctly points out that the

Applicants wished to claim a method in which the nucleic acid encodes a fusion peptide, and suggests that clarification is appropriate. Accordingly, Applicants have amended claim 3 replacing "comprises" with "encodes". Additionally, Claims 4 and 5 are amended to replace "peptide" with "protein".

Claims 32 and 33 were objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim (claim 30) and any intervening claims. Accordingly, Applicants have submitted new claims 37-42. Claim 37 corresponds to claim 32, rewritten in independent form. Claims 38 and 39 correspond to rejected claims 31 and 34, respectively, now depending from allowable claim 37. Claim 40 corresponds to claim 33, rewritten in independent form. Claims 41 and 42 also correspond to rejected claims 31 and 34, respectively, but here depending from allowable claim 40.

Applicants respectively submit that the present amendment avoids the rejections of the last Office Action and places the application in condition for allowance or appeal. The amendment introduces no new subject matter and does not present any new issues requiring further consideration or additional searching. The amendment presents additional claims, but the new claims 37 and 40 correspond to objected to claims that have been rewritten in independent form, pursuant to the Examiner's suggestion. Applicants respectfully urge that new claims 38, 39 ,41 and 42 are in condition for allowance, as they depend from allowable claims. Applicants respectfully request reconsideration by the Examiner of claims 30- 34 in light of Applicants' remarks herein regarding the Hartley '732 reference.

CONCLUSION

Applicants respectfully believe that Claims 1- 20, 26, 30-35 and 37-42 are now in condition for allowance. Applicants respectfully request the approval of the Examiner for the present amendment after final and advancement of the application to allowance. Applicant respectfully submits that no fees are due. If this is incorrect, the Commissioner is hereby authorized to charge any fees which may be required by this paper to Deposit Account 07-0153. Accordingly, a favorable action in the form of an early notice of allowance is respectfully requested. The Examiner is requested to call the undersigned for any reason that would advance the instant application to issue.

Dated this the 3th day of April, 2000.

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